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BIOACTIVITY ASSESSMENT OF POMEGRANATE

Hina gul[1], Aafia Islam[1], Hania Naheem[1] , Narmeen Arif[1], Farhana BiBi[2], Waqas Ahmad[2] and Muhammad Gulfraz[2]

1- University Institute of Biochemistry and Biotechnology, PMAS Arid Agriculture University Rawalpindi, Pakistan
2- Grand Asian University Islamabad

***Corresponding Author:** gul.fraz@gaus.edu.pk

Pomegranates is useful for human health due presence of bioactive nutrients. Modern science has provided evidences that regular uses of pomegranates can reduce risk of heart stocks.

In this study, extracts of pomegranate seeds and peels were evaluated for their potential antioxidant and cytotoxic effects, and the findings of these evaluations were linked using an in-silico model. Chemical analysis of the extracts was used to identify the total phenols, flavonoids, tannins, vitamin C, thiamine, riboflavin, and a few essential metals. Also determined were the total tannins. According to the results of an HPLC analysis, pomegranate extracts have a higher quercetin content than the fruit itself. Flavonoids, vitamins, and critical metals were all shown to have a strong correlation with oxidative stress indicators and cytotoxicity tests when they were examined in extracts. The findings of an experiment conducted on animals in which the effects of antidepressants were simulated indicated that pomegranate extracts helped worried animals feel better in a shorter amount of time. In addition, the function of quercetin 3-glucosides as a ligand was identified in a computer-simulated experiment by docking with the 2-androgenic protein receptor, which is a receptor for the hormone adrenaline. This function was discovered when the quercetin 3-glucosides interacted with the receptor. It is possible that the epinephrine blood pressure of people may be lowered by decreasing the effects of stress.

Keywords: Pomegranate, Chemical analysis , Antioxidants, ,Silico study

INTRODUCTION

Punicagranatum L., which is linked to the punica family and is known as one of the oldest edible fruits, was named after the genus Punica (Singh, 2002). (Singh, 2002). The pomegranate fruit has been used for therapeutic purposes since ancient times. It is also utilised in the production of fresh juices and beverages, as well as jam and jelly, and as a flavouring and colouring reagent in the food industry (Gurib-Fakim, 2006). Even when ingested in big amounts, preliminary toxicological studies indicate that pomegranate is not harmful to human health and may be safely consumed in any amount. Pomegranate tree bark, roots, and leaves are all useful components of a therapeutic remedy. The peel extracts contained a higher quantity of ellagic acid and ellagic tannins, which are two of the most effective antioxidants known, and the peel extracts also had ellagic tannins ( ). Ellagitannins have the potential to be used in the production of natural antibiotics due to their efficiency in destroying Staphylococcus aureus and other potentially harmful bacteria (Bekiret al., 2013). In addition to the treatment of infections of the male and female genital organs, mastitis, acne, piles, allergies, and dermatitis, pomegranate has been said to have antiproliferative properties on breast cancer cells known as MCF-7 (Chandra et al., 2010). Changes in hormone levels and the activity of enzymes have provided evidence to support the assertion that it is effective in combating the negative effects of stress. It has been shown that excessive stress has considerable effects on both behavioural and learning processes, as well as physiological systems, which may result in depression (Gullon et al., 2016). It is possible to pass away from major depressive illness as well as other mood disorders. It is possible for antidepressant drugs to induce unwanted side effects or to have a diminished impact on the desired outcomes (Kharachoufi et al., 2018). Because of the high quantities of polyphenols, vitamin C, and macro elements found in pomegranate seeds and peel, pomegranate has been shown to possess antioxidant capabilities. Other macro elements found in pomegranate include potassium, calcium, magnesium, iron, zinc, and manganese (Aviram et al., 2008; Tehranifar et al.,2010). Through the use of molecular docking, the current research sought to investigate whether or not there is a connection between the phytonutrients found in the peels and seeds of pomegranate fruit and a reduction in the levels of hypertension experienced by the participants.

MATERIALS AND METHODS

Sample collection and preparation

The neighbourhood of Kotli Sattian in Rawalpindi was searched thoroughly for its supply of fresh pomegranate (punica granatum L.) fruit (Pakistan). The UIBB PMAS Arid Agriculture Rawalpindi laboratory conducted the analyses of all of the samples during the years 2020 and 2021. A voucher specimen of the plant, with the number 136, has been archived at the Herbarium of the Department of Botany for the benefit of researchers in the future. The seeds and peels of pomegranates were first sun-dried, then dried in an oven at 60 degrees Celsius, and then shad-dried. The samples were ground into powder using a sieve as the grinding apparatus (80 msh). To extract a total of 100 grammes of dried material, the soxhlet and rotary evaporator procedures were used, along with distilled water, methanol, ethanol, and chloroform. mathematical modelling and simulation

The total phenol, flavonoid, and tannin content of extracts of pomegranate peel and seeds were determined by Folin's Ciocaletu as well as other colorimetric methods (Harbone, 1984; Abbasiet al., 2015). Oil was extracted from pomegranate samples by Soxhlet techniques, which included the use of methanol, ethanol, and chloroform. The spectrophotometric method was used to determine the vitamin concentrations. The levels of sodium, potassium, calcium, and magnesium, in addition to the levels of the micronutrients iron, zinc, and manganese, were tested in accordance with a technique that used atomic absorption spectroscopy (AOAC, 2000).

High-performance liquid chromatography was used using a Shimadzu HPLC system that was located in Tokyo, Japan. This system was outfitted with a UV/visible detector and a C18 column that measured 25mm 4.5mm and 5 metres in length (HPLC). For the purpose of eluting the compounds, acetonitrile and 0.1% phosphoric acid were used (36:64). The volume of injection was 20 l for each of the samples. A 280 nm and a 285 nm detector were used to monitor the flow of flavonoids at a rate of one millilitre per minute. The quercetin concentration served as the benchmark for all of the measurements, which were all carried out in triplicate.

Brine shrimps cytotoxic assay

The previously reported brine shrimp cytotoxicity assay was used in this study to evaluate the cytotoxic potential of pomegranate seed and peel extracts (Ruchet al.,1989).

Determination of antioxidant capacity of extracts in vitro

DPPH radical scavenging activity was assessed, as was previously mentioned (Moon andShibamoto, 2009; Yu et al.,2005). A determination made with the use of 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) (Ashafa et al.,2010). In order to investigate the effects of hydroxyl radicals, superoxides, and iron chelation, a method that has been published was used (Cefarelliet al.,2006). To get the IC50 values, the dose response curve was used.

Depression study

In order to carry out this research, we went to the National Institute of Health in Islamabad and purchased 18 albino mice of both sexes with a total weight of 52.51.6g. These mice would serve as our animal subjects. As is typical procedure at a research facility, each animal was housed in its own individual cage and allowed unlimited access to the laboratory's supply of water and food pellets. Before giving their approval for the behavioural trial and research, the ethics committee that the university had formed for conducting experiments on animals required a three-day acclimatisation period as a prerequisite. It was determined how immobile the animal was based on how well its tail was suspended (Steru et al., 1985). A momentary sagging was created by the tape that was draped from the animal's tail. During the period of the test, which lasted for a total of 6 minutes, the participant was instructed to maintain complete and utter stillness. The length of time in which neither limbs or the body move at all is referred to as the immobility phase (Except movement caused by breathing). The following drugs were tested to see how long animals could stay motionless after being given them, and the results are presented below.

Experimental design

Randomization was used to divide the animals into six groups of three each. As a placebo, one millilitre of salt water containing 0.9% sodium chloride was mixed into each animal's diet that was part of Group I. Fluoxetine at a dose of 20 mg/kg was administered to the animals in Group 11, which served as a positive control for the study. Group III was given a dosage of

methanolic peel extracts that was equal to 100 mg/Kg body weight. The people who were in group IV were given methanolic peels at a rate of 200 milligrammes for every kilogramme that their bodies weighed. Animals in group V were administered methanolic seed extracts at a dose of 100 mg/kg of their body weight. Group VI was administered extracts of seeds that had been methanolically treated at a dosage of 200 milligrammes per kilogramme of body weight. Intraperitoneally (i.p.) administration of fluoxetine, a common therapy for depression, was performed on animals while gavage administration of methanolic extracts was carried out.

Molecular docking

We compared the structure of the beta 2-adrenergic protein receptor to the structure of the quercetin 3-glucosides ligand by using the Vina suit (PyRx, version 0.8), as well as the Automatic server Patch dock (online server). The structure of the receptor was obtained by downloading it from the Protein Data Bank (PDB), while the structures of the ligands were obtained by downloading them from Pub Chem and then transferring them to PDB using PyMol. The Avogadro tool was used for receptor surface analysis and docking in order to minimise the amount of energy lost while maximising the amount of geometric accuracy. Lig Plus was used for H bonds and hydrophobic interactions, while Chimera version 1.10.2 was used for docking receptor ligands. Both programmes were used in this study (Morris et al., 2009).

Statistical analysis

Following the completion of three separate investigations, the resultant data was subjected to statistical analysis in order to identify key patterns and outliers.

RESULTS AND DISCUSSION

Chemical analysis of pomegranate extracts

Pomegranate peel extracts have led to higher yields of total oils, while methanolic seed extracts have provided higher amounts of total phenolics, total flavonoids, and tannins. Both of these results may be attributed to the pomegranate fruit (Table 1). Table 2 presents the results of an investigation into the water-soluble vitamin, macronutrient, and micronutrient composition of extracts of peel and seeds.

Brine shrimps lethality assay

The cytotoxic effects of pomegranate seed and peel extracts were investigated using brine shrimp in a research with four different concentrations of the extracts (100, 200, 400, and 600 g/mL) (Table 3). At the highest possible concentration of the extracts, the larvicidal capability and lethality of the enhanced brine shrimp were found to be at their peak. It was hypothesized that the extracts included phytonutrients of essential importance, many of which had anticancer properties.

Tables 1. Quantification of phytochemicals from Seeds and Peels extracts of pomegranate

Extracts	**Total flavonoids (mg Quercetin /100g)**	**Total phenols (mg Caffeic acid /100g**	**Tannins (mg/100g)**	**Oil (%)**
Methanol seed extract	47.12 ± 1.32	215.31 ± 1.23	2.95 ± 0.58	3.4 ± 0.3
Ethanol seed extract	41.15± 1.02	218.74 ± 2.35	3.53± 0.63	3.5 ± 0.5
Chloroform seed extract	36.34 ± 0.63	216.25 ± 2.7	2.85 ± 0.52	2.4 ±0.3
Methanol peel extract	37.16 ± 1.53	124.35 ± 1.26	3.67 ± 0.56	4.3 ±0.4
Ethanol peel extract	36.12± 1.64	114.75 ± 1.35	3.24± 0.63	4.4±0.6

Chloroform peel extract	25.35 ± 0.53	112.23 ± 1.56	2.32 ± 0.56	2.5 ± 0.5

Mean ± SD (n=3).

Table 2. Concentration level of Vitamins (mg/dL) and Metals ions (%) in peels and seed extracts of Pomegranate.

Analytes	**Peel extract**	**Seed extract**
Ascorbic acid	8.82 ± 0.56	9.73 ± 0.75
Thiamine	0.18± 0.61	0.43 ± 0.02
Riboflavin	0.16 ± 0.53	0.12 ± 0.01
Sodium	2.67± 0.56	3.07± 0.28
Potassium	205.6± 0.13	225.3± 0.18
Magnesium	11.84 ±1.46	12.34 ±1.24
Calcium	8.15 ± 0.62	9.76 ± 0.35
Iron	0.46 ± 0.04	0.67 ± 0.05
Znic	0.64 ± 0.01	0.89 ± 0.04
Manganese	0.15 ± 0.02	0.18 ± 0.05

Mean ± SD (n=3)

Table 3. Cytotoxicity screening of various concentration (µg/ml) extracts of pomegranate and % mortality.

Extracts	**100**	**200**	**400**	**600**	**LD_{50}**

	mg/ml	**mg/ml**	**mg/ml**	**mg/ml**	
Methanol pees extract	40	50	60	80	270.45
Etanolic peels extract	50	60	80	90	16.27
Chloroform peels extract	55	70	70	70	324.82
Methanolic seeds extract	10	30	50	60	14.77
Ethanolic seeds extract	20	25	55	65	319.77
Chloroform seeds extract	30	30	60	70	372.53

Values are Mean ±SD (n=3) and significantly different (P<0.05);positive control are saline sea salt.

Antioxidant capacity in vitro

It was discovered how successful each of these five distinct methods were in determining the levels of antioxidant activity contained in pomegranate extracts. In spite of this, the results of the DPPH test for the free radical scavenging activity of the methanol seed extract were the most encouraging (IC50 = 15.291.49g/mL) (Table 4). The DPPH test is a very accurate method for determining the level of antioxidant capacity possessed by biological substrates. Flavonoid is only one of numerous chemical components that may be present in seed extracts; the synergistic

action of these other components may be to blame for the increased antioxidant capacity of the extracts (quercetin)

Table 4.Antioxidant effects of peels and seeds extracts of Pomegranate (IC50 values µg/mL)

Extract 100 µg/mL	**DPPH**	**H_2O_2**	**ABTS**	**Reducing power**	**Iron chelation**
Ethanolic peel extract	28.25±2.21b	44.51±1.18b	42.92±1.51b	66.03± 2.14b	36.31± 1.38b
Methanolic peel extract	16.28±1.45a	38.25±2.17$^{\alpha}$	39.52±2.31$^{\alpha}$	63.19± 2.54 a	25.52± 2.34 a
Aqueous peel extract	36.35±0.48	46.62±2.81	47.21±1.73	71.45± 2.66	46.09± 1.17
Ethanolic seed extract	26.19±1.28b	43.56±2.18b	45.72±3.81b	68.53± 2.06b	36.32± 1.35b
Methanolic seed extract	15.29±1.49a	41.22±2.15$^{\alpha}$	38.52±2.33$^{\alpha}$	61.19± 3.15 a	24.65± 1.36 a
Aqueous seed extract	34.13±2.11	52.43±3.45	46.21±2.31	92.16± 3.64	41.35± 1.27
Ascorbic acid	8.25±0.72b	7.52 ±0.39b	6.34±0.35 Þ	11.35 ±1.17 Þ	35.06± 1.28b
Gallic acid	3.93±0.38 a	1.68±0.23 a	1.23±0.34 a	2.35±0.38 a	20.38±1.38 a

Means ± SD, (n = 3), whereas $^{\alpha}$ = $p<0.01$,Þ= $p<0.05$; a higher values , b lower values

In Vivo antidepressant study

According to the data, there were no fatalities even at the maximum possible doses of the medicine after 72 hours had passed. Extracts at 100 mg/kg had no significant influence on

immobility time, however extracts from seeds, especially those extracted in methanol, demonstrated powerful antidepressant effects that were equal to the conventional medicine fluoxetine when administered at 200 mg/kg (Table 5). The tail suspension test is often used in the testing of new antidepressants (TST).

Table 5.Antidepressant like activity of seeds and peels extracts on immobility period (second) of rats using tail suspension test.

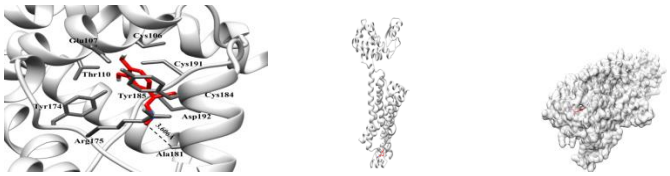
Group	Drugs	Pre treatment	Post treatment
1	Negative control (saline 0.9 %)	195.4 ± 2.5	193.5±2.7
2	Positive control Fluoxetine (20 mg/kg)	185.8 ±1.6	175.6 ±1.6 ^{S}
3	Methanolic peels extract 100 mg/kg	189.6 ±1.3	184.5 ±1.7
4	Methanolic peels extracts 200 mg /kg	187.5 ±2.6	181.6 ±2.1
5	Methanolc seeds extracts 100 mg/kg	185.7 ±1.5	179.5 ±1.8 ^{B}
6	Methanolic seeds extracts 200 mg/kg	182.6±2.6	176.8 ± 2.4 ^{A}

Values are Mean ± SD (n=3). Higher effects methanolic seed extracts (A and B) as compared to standard drugs (S) to overcome depression condition of animals.

Molecular docking

In order to create a ligand, quercetin glycosides were synthesised. Then, the polar interactions that occurred between the receptor and the ligand were analysed in order to find the

conformation of the ligand that was most effective in activating the alpha-2 noradrenergic receptor (Fig.2 ABC). According to the results of the docking simulation, the ligand probably interacts with the active sides of the receptor at Glu 107, Cyst 106, 184 and 191, Thr110, 174 and 185, Arg 175, Ala 181, and Asp 192. The interaction between a ligand and a protein cavity may either further activate or inhibit the protein depending on whatever state the protein is in. When the neurotransmitter epinephrine binds to the beta-2 adrenergic receptor, also known as ADRB2, a cascade of physiological events takes place. One of these reactions is most likely the relaxation of smooth muscles. According to the findings of certain research, a reduction in blood pressure and adrenaline levels may be achieved by the intake of quercetin, vitamin C, and other macronutrients such as potassium and calcium, which can be found in fruits and vegetables. That, as a result, reduces the amount of anxiety and despair that the affected person experiences and provides relief from the existing state of stress.



A B C

Figure 1. Model of molecular docking

According to the findings of the current research, pomegranate seed and peel extracts have a lower degree of cytotoxicity than other plant materials, making them appropriate for use in the manufacturing of pharmaceuticals. Furthermore, the promising level of antioxidant activities of seed and peel extracts demonstrates a link between flavonoids, phenolics, and tannins. These constituents make up tannins. It was discovered that quercetin was the most abundant component, and it is very likely that tannins, namely ellagic tannins, are the primary antioxidants.

Although oil quantification investigated the potential health benefits of pomegranate fruit, which may include both essential and non-essential fatty acids, the fruit itself was not of much importance to the investigation. It is possible that the antidepressant-like effects of pomegranate

extracts are attributable to the presence of water-soluble vitamins (such as vitamin C, thiamine, and riboflavin), as well as micro and macro nutrients. These vitamins and nutrients have been shown to have a positive impact on muscular relaxation and to help reduce anxiety levels in effective subjects.

However, the damaging effects of reactive oxygen and nitrogen species may be minimised by ingesting foods high in antioxidants, especially fruits and vegetables. Oxidative stress has been linked to a wide range of illnesses and health issues in humans (Sing et al.,2002; Yu et al., 2005). The antioxidant potential of pomegranate extracts, as shown in this study, is comparable to that discovered in other investigations by other authors (Negiand Jayaprakasha, 2003;Elfalleh et al. 2012).

According to the results of our research, extracts made from pomegranates may have both antidepressant and antioxidant characteristics. Oxidative stress may cause cancer and other illnesses, and vitamin C, potassium, and calcium all have biochemical properties that make them potentially helpful in protecting against these conditions (Morris et al., 2009). There are a number of potential causes for symptoms of anxiety and depression, some of which include hypertension, the environment, and heredity. Fruits high in flavonoids, vitamin C, and essential metals should be consumed on a regular basis to achieve the potential antidepressant benefits of these foods.

Researchers were able to identify potential hydrophobic and hydrophilic sites for interaction between tiny molecules (ligands) and protein at the atomic level by making use of a technique called molecular docking. These sites were found in the region where quercetin was bound to the beta -2 adrenergic receptor (Hinaet al., 2017). As a result of this, eating fruits that are high in flavonoids (Quercetin) has the potential to lower the levels of factors that induce stress and high blood pressure (Acharya et al., 2010).

CONCLUSION

The pomegranate is an important fruit because of the high concentration of flavonoids (quercetin), water-soluble vitamins (vitamin C), essential fatty acids, and metals that it contains. These constituents all work together to provide antioxidant protection, slow the development of cancer cells, and possibly even alleviate depression. We discovered that consuming fresh

pomegranate fruits has a variety of positive effects on one's health, which has led to the fruit's broad use in complementary and alternative medicine.

REFERENCES

Abbasi AM., Shah MH., Li T., Fu Guo X and Liu RH. (2015). Ethnomedicinal values, phenolic contents and antioxidant properties of wild culinary vegetable. Journal of Ethnopharmacology ,

162; 333-345

Acharya A., Das IS., Singh S and Saha T.(2010). Chemopreventive properties of indole-3-carbinol diindolylmethane and other constituents of cardamom against carcinogenesis. Patents on Food, Nutrition and Agriculture, 2; 166-177.

AOAC. (2000). Official Methods of Analysis of the Association of Official Analytical Chemists, (17th . ed) Washington Dc.

Ashafa AOT., Sunmonu TO and Afolayan AJ. (2010). Food and Chemical Toxicology ,48; 1886-1889.

Aviram M., Volkova N., Coleman R., Dreher M., Redd MK., Ferreira D and Rosenblat M. (2008) . Toxicological evaluation of aqueous leaf and berry extracts of Phytolaccadioical in male Wistarrats. Pomegranate phenolics from the peels, arils, and flowers are antiatherogenic: studies in vivo in atherosclerotic apolipoprotein E- deficient (E0) mice and in vitro in cultured macrophages and lipoproteins. Journal of Agriculture and Food Chemistry, 56; 1148-1157.

Bekir J., Mars M., Souchard JP and Bouajila J. (2013). Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (Punicagranatum) leave. Food and Chemical Toxicology, 55; 470-475.

Cefarelli G., Abrosca BD., Fiorentino A., Izzo A., Mastellone C., Pacifico S and Piscopo V. (2006). Free-radical-scavenging and antioxidant activities of secondary metabolites from reddened cv. Annurca apple fruits. Journal of Agriculture and Food Chemistry, 54; 803-80.

Chandra R., Tejrao V and Sharma J. (2010). Global scenario of pomegramate (Punica granatum L.) culture with special reference to India. Fruit Vegetable and Cereal Science and Biotechnology, 4(2); 7-18.

Elfalleh W., Hannachi H., Tlili N., Yahia Y., Nasri N and Ferchichi A. (2012). Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. Journal of Medical Practice and Reviews, 6 ;4724-4730.

Gullon B., Pintado M., Pérez-Álvarez J and Viuda-Martos M. (2016). Assessment of polyphenolic profile and antibacterial activity of pomegranate peel (Punica granatum) flour obtained from co-product of juice extraction. Food Control, 59;94-98

Gurib-Fakim A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27; 1-93.

Harborne JB. (1984). Phytochemical methods: A guide to modern techniques of plant analysis Chapman and Hall London.

Hina G., Mushtaq A., Zafar M., Muhammad SA., Ambreen A., Syeda H., Imam S and Gulfraz M. (2017). The invitro and invivo Biological activities of the leaf of Cape MyrtleMyrsine Africana L. Phytotherapy Research, 31; 1305-130.

Kharchoufi S., Licciardello F., Siracusa L., Muratore G., Hamdi M and Restuccia C. (2018). Antimicrobial and antioxidant features of “Gabsi” pomegranate peel extracts. Industrial Crops and Products, 111; 345-352

Li Y., Guo C., Yang J., Wei J., Xu J and Cheng S. (2006). Evaluation of Antioxidant Properties of Pomegranate Peel Extract in Comparison with Pomegranate Pulp Extract. Food Chemistry, 96; 254-260.

Miguel G, Fontes C, Antunes D, Neves A, Martins D. 2004. Anthocyanin concentration of "Assaria" pomegranate fruits during different cold storage conditions. BioMed Research International, 338-342.

Moon JK and Shibamoto T. (2009). Antioxidant assays for plant and food components. Journal of Agricultural and Food Chemistry, 57; 1655-1666.

Morris GM., Huey R., Lindstrom W., Sammer MF., Belew RK., Goodsell DS and Olson AJ. (2009). Auto Dock 4 and AutoDock Tools 4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry ,30; 2785-2791.

Murthy KC., Jayaprakasha G and Singh R. (2002). Antioxidant Activity of Pomegranate Peel Extracts in Vivo Models. Journal of Agricultural and Food Chemistry, 50; 4791-4795.

Negi P and Jayaprakasha J.(2003) Antioxidant and Antibacterial Activities of Punicagranatum Peel Extracts. Journal of Food Sciences, 68; 1473-1477.

Reddy MK., Gupta SK., Jacob MR., Khan SI and Ferreira D. (2007). Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from Punicagranatum L. Planta Medica, 53; 461-467.

Ruch RJ., Cheng SJ and Klaunig JE. (1989). Prevention of cytotoxicity and inhibitionof intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10; 1003-1008.

Singh RP., Chidambara Murthy KN and Jayaprakasha GK. (2002). Studies on the antioxidant activity of pomegranate (Punicagranatum) peel and seed extracts using in vitro models. Journal of Agricultural and Food Chemistry, 50; 81-86.

Steru L., Chermat R., Thierry B and Simon P. (1985). The tail suspension test : A new method for screening antidepressant in mice. Psychopharmacology, 85; 367-370.

Tehranifar A., Zarei M., Nemati Z., Esfandiyari B and Reza M. (2010). Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (Punica granatum L.) cultivars. Scientia Horticulturae, 126(2); 180-185

Yu J., Wang L., Walzem RL., Miller EJ., Pike LM and Patil BS.(2005). Antioxidant activity of citrus limonoids, flavonoids and coumarins. Journal of Agricultural and Food Chemistry , 53; 2009–2014.